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Effect Of Arginine On The Some Blood And Biochemical Parameters For Local Goat Females.

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Abstract

This study was conducted in one of the private sector fields in Anbar province - Hit city - Albasaer village (70 km west of the Ramadi city,), for the period from 28/6/2018 to 1/9/2018. Twenty one local female goats aged between 2-4 years and weighing between 24.5 to 36.5 kg were used, Which have previous one birth or more. All female goats were tested using ultrasound to make sure they were not pregnant before the experiment began. Females were randomly divided into three equal groups (7 goats in each group).Vaginal sponges (60 mg MAP) were injected into the three groups at the same time. The first group T1 was injected intramuscularly with the amino acid, arginine (US Nevada manufacturing) in the muscles at 200µmol.kg Five days before the sponge was pulled out until the 17 day after the sponge was pulled out, While the second group T2 was injected with amino acid (arginine) at 160 µmol.kg. Five days before the sponge was pulled until the 17th day after that. The third group T3 control group was injected with 5 ml Normal Saline intramuscularly of the animal. All animals were injected three times daily from the eighth day after the sponge was placed (five days before removing of the sponge) until 17 days after the removing of sponge. Where the total number of injection days was 22 days. Blood samples were taken from the jugular vein before injection of the arginine on day 7 and day 12 of the sponge placed either after the sponge pulled the blood samples were taken on the days 2, 3, 4, 6, 9, 13, 18, respectively of the experiment After sponges removing. The objective of the study was to measure changes in the blood, biochemical parameters, during arginine treatment. The results of this study showed asignificant differences. T1 and T2 group were superior compared to control group in blood properties which include pcv in periods 2, 5, the number of white blood cells in periods 2, 3, 4, 5, 6, 9, MID in periods 3, 9 and Lymphocytes in aperiods 4, 5, 6, 9, While the neutrophil cells the period of 3. In terms of biochemical properties, the results showed asignificant differences, between the treatments of T1 and T2 were superior compared to control treatments in the total protein concentration in period 6, and the globulin in period 6. We conclude that the use of different doses of arginine can improve the health status of female goats.

تأثير الآرجنين في بعض الصفات الدمية والكيماحيوية لإناث الماعز المحلية

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الخلاصة

أجريت هذه الدراسة في احد حقول القطاع الخاص في مدينة هيت- قرية بصائر(غرب مدينة هيت) التابعة لمحافظة الأنبار 70 كم غرب مدينة الرمادي، العراق، للفترة من 2018/6/28 ولغاية 2018/9/1. استخدمت 21 أنثى ماعز محلية بعمر تراوح بين 2-4 سنوات وذات وزن يتراوح بين 24,5-36,5 كغم، وذات ولادة واحدة على الأقل فحصت جميع إناث الماعز باستخدام جهاز الموجات فوت الصوتية (السونار) للتأكد من خلوها من الحمل قبل بداية التجربة. قسمت الإناث عشوائيا الى ثلاث مجاميع متساوية (7 معزات تصنيع الارجنتين) للمجاميع الثلاثة في نفس الوقت، المجموعة الأولى MAPفي كل مجموعة) تم دفع الاسفنجات المهبلية (60 ملغم kg/µmol الامريكية) في العضل بمقدار Nevada 200 تم حقن اناث المجموعة بالحامض الاميني الآرجنين (تصنيع شركة T1 تم حقن اناث المجموعة بالحامض T2قبل خمسة ايام من سحب الاسفنجات ولغاية يوم 17 من سحب الاسفنجة. اما المجموعة الثانية

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T2 تم حقن اناث المجموعة بالحامض الأميني الآرجنين في العضل بمقدار 160 kg/µmol. قبل خمسة ايام من سحب الاسفنجات ولغاية يوم 17 من سحب الاسفنجة. اما المجموعة الثالثة T3 معاملة السيطرة تم حقنها بمحلول الملح الفسلجي بمقدار 5 مل (Normal Salin) في عضل الحيوان. تم حقن جميع الحيوانات بواقع ثلاث مرات يوميا من اليوم الثامن بعد دفع الاسفنجة (قبل خمسة من سحب الاسفنجة) ولغاية يوم 17 بعد سحب الاسفنجة حيث يكون مجموع ايام الحقن الكلي 22 يوم. تم سحب عينات الدم من الوريد الوداجي في اناث التجربة قبل حقن الآرجنين في اليوم 7 واليوم 12 من دفع الاسفنجة اما بعد سحب الاسفنجة سحب الدم في الايام 2، 3، 4، 6، 9، 13، 18 على التوالي من التجربة بعد الازالة. كان الهدف من هذا لقياس التغيرات في الصفات الدمية والكيماحيوية ومجموعة الدهون اثناء المعاملة بالآرجنين. اذ اظهرت نتائج الدراسة وجود فروق معنوية اذ تفوقت T1 و T2 على السيطرة في الصفات الدمية التي شملت كل من حجم الخلايا المرصوصة في الفترات 2، 5، واعداد كريات الدم البيضاء في الفترات 2، 3، 4، 5، 6، 9. و (MID) في الفترات 3، 9. والخلايا اللمفاوية (Lymphocytes) في الفترات 4، 5، 6، 9. اما الخلايا العدلة (Neutrophil) في الفترة 3. وفيما يخص الصفات الكيماحيوية اظهرت النتائج فروق معنوية اذ تفوقت T1 و T2 على السيطرة في تركيز البروتين الكلي في الفترة 6، والكلوبيولين في الفترة 6. نستنتج من الدراسة الحالية ان استعمال جرع مختلفة من الارجنين قد حسنت الحالة الصحية لإناث الماعز.

الكلمات المفتاحية : ارجنين ، الصفات الكيماحيوية ، كريات الدم البيض

Keywords: Arginine, Biochemical Parameters, WBC.

Introduction

The food security is one of the main objectives that producers now aspire to. The importance of livestock production and other products is to balance actual production and nutritional needs in any country, so there is a real need to develop the productive sector to meet the challenges of increasing demand. Therefore, many researchers in their studies are interested in an increasing the production by increasing the number of animals and the increase in comes through raising the rate of immunization to diseases and reduce their mortality for agood levels(1). The researchers were interested in studying dietary supplements containing amino acids and mineral salts. The amino acid arginine is almost essential for adult ruminants, so it requires large and small animals. Arginine is the only important physiological unit for the synthesis of nitric oxide (NO) and polymer (2). Both are essential for the proper development of animal health and growth (3). Arginine is also one of the functional amino acids in protein synthesis (4). There are indications that dietary supplements or injections of the amino acid arginine are effective within the body. It improves reproductive function, cardiovascular function, immunity and tissue integrity (5). (3) concluded that the use of arginine promotes early uterine environment, making it ideal for fetal survival and maintenance of pregnancy, and regulation of food metabolism and immune response (6). It also works to protect cells from with arginine increases fetal growth by increasing the processing of nutrients to the fetus (8). In view of the importance of the amino acid arginine in the improvement of immunological properties and chemical properties and the lack of studies in this aspect in Iraq, the study aimed to study the effect of arginine on the some blood and biochemical parameters of local females goat.

Materials and Methods

This study was conducted in a private field in Anbar province - Hit city - Albasaer village (70 km west of the Ramadi city), from 28/6/2018 to 1/9/2018. 21 female non-pregnant goats were used depending on their physical condition, age and weight, They were in good physical condition with at least one birth and with an age of 2-4 years and an average weight of 24.5-36.5 kg. All female goats were examined using the ultrasound device (Carelive cd66v, Chinese Manufacturing). Before by using the vaginal sponges, to ensure that they were free of pregnancy before the start of the experiment, the female goats were randomly divided into three equal groups (7 goats in each group). The females were treated three times a day. The dividing of groups was as follows:

The first group T1 was injected with the arginine (US Nevada manufacturing) in the muscles at 200µmol/kg Five days before the

sponge was pulled out until the 18th day after the sponge was pulled out, While the second group T2 was injected with arginine at 160 µmol/kg. Five days before the sponge was pulled until the 18th day after that. The third group T3 control treatment was injected with 5 ml Normal Salin in muscles of the animal. The mating process was conducted by placing males with females after removing the sponges and without injecting the hormone for 5 days. The males were rotated between the cages to eliminate the effect of the male differences. Blood samples were taken from the jugular vein of females before injection of the arginine on day 7 and day (12) of the sponge placed either after the sponge pulled the blood samples were taken on the days 2, 3, 4, 6, 9, 13, respectively of the experiment after sponges removing. To measure changes in levels of blood parameters (WBC, number of White blood cells, PCV) and biochemical parameters (Total protein, Albumin, Globulin). One-way analysis was conducted included effects of arginine and periods. Using the General Linear Model and the SAS Statistical program Edition 9.1 (9). The significant differences among the averages were tested using a Polynomial test (10) to determining each of WBC lymphocyte, Monocyte, Neutrophil using the Complete Blood picture (genex type American manufacturing, 2012). Globulin was estimated by the following formula according to (11) (12).

Globulin = Total Protein – Albumin

The total protein was estimated according to the instructions of manufacturer and the method of (13). The albumin was also estimated according to the instructions in the work tools and based on the method of (14).

Results and discussion

Effect of arginine on blood properties:

The results of Table 1 showed significant differences in the size of the backed cells (PCV), where T1 group was superior compared to the control group in period 2, 5 and group of T2 was superior compared to control group in period 2, while the other periods showed no significant differences, this superiority may be attributed to the role of arginine in improving the growth and development of body cells by increasing the blood circulation of organs (15), and this leads to healthy indicators have been observed compatibility between the improvement of the reproductive situation with health indicators improving, through the increase of nutrients in the blood. Some studies point to the role and importance of weight and its effect on the size of packed cells where the PCV is increased by weight increase (16)(17). Also the results of Table 2 showed no significant differences in the number of white blood cells among the treatments of T1 and T2 and control in periods 1, 7 and 8 with significant differences in WBC numbers in the period 2, 3, 4, 5, 6, and 9 where the WBC numbers in goats blood increased in the arginine groups compared to control group. This results in agreement with results of (18) which were observed that addition of arginine has led to the improvement of cellular immunity and humoral immunity and production regulation of Leukocytes and Antibodies. The results of his study showed an increase in the numbers of WBC in small pigs treated with arginine compared to the control group by modifying the production of W.B.C. The higher values of blood parameters such as W.B.C number in T1 and T2 compared to control groups due may be to the role of arginine, which stimulates the secretion of growth hormone and insulin, which improves immune response (19). Also (6) noted that sufficient arginine is necessary for the development of lymphocytes and that dietary arginine supplementation enhances immune function in different models of immunological challenges. The oxidative stress weakens the response of the immune system to reduce the immune response in order to prevent reactive oxygen types in cells. It also leads to physiological changes between the immune system and antioxidant, as arginine acts as an antioxidant and the negative impact of oxidative stress on immunity can help prevent any Inhibition of the expected immune response after immunosuppression and immunization (20).

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Table – 1: Effect of arginine on (PCV) in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *1.25 ± 24.4	A 0.808 ± 23.2	A 1.75 ± 22.0	N.S.**
2	ABC 0.993 ± 22.7	AB 0.528 ± 22.5	A 1.32 ± 19.2	0.0437
3	ABC 1.10 ± 22.5	B 0.571 ± 20.5	A 1.20 ± 19.2	N.S
4	AB 0.961 ± 24.1	A 0.459 ± 23.1	A 1.49 ± 20.5	N.S
5	A 0.420 ± 25.2	A 0.828 ± 24.1	A 1.48 ± 21.1	0.0266
6	ABC 0.769 ± 23.1	A 0.723 ± 24.0	A 1.38 ± 22.0	N.S
7	BC 0.996 ± 21.4	A 1.22 ± 24.1	A 1.06 ± 20.4	N.S
8	C 0.611 ± 20.4	B 0.704 ± 20.8	A 0.808 ± 19.2	N.S
9	C 0.368 ± 20.4	B 0.521 ± 20.7	A 1.34 ± 19.0	N.S
Significant Level	0.0012	0.0007	N.S	

* Values=Means ± SE

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within a row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

Table – 2: Effect of arginine in WBC in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *2.11 ± 13.9 a	A 1.48 ± 10.4 a	A 0.586 ± 9.95 a	N.S**
2	A 1.34 ± 13.7 a	A 1.55 ± 12.7 a	A 0.566 ± 8.71 b	0.0230
3	A 1.40 ± 12.9 a	A 1.35 ± 10.3 ab	A 0.721 ± 7.97 b	0.0307
4	A 1.67 ± 14.4 a	A 1.30 ± 11.5 ab	A 1.03 ± 8.90 b	0.0333
5	A 0.965 ± 14.4 a	A 1.10 ± 11.9 ab	A 0.931 ± 10.3 b	0.0327
6	A 0.989 ± 12.8 a	A 0.802 ± 10.7 ab	A 0.644 ± 8.90 b	0.0124
7	A 1.58 ± 12.7 a	A 1.42 ± 12.1 a	A 1.03 ± 10.2 a	N.S
8	A 1.10 ± 11.5 a	A 0.977 ± 10.0 a	A 0.992 ± 9.12 a	N.S
9	A 0.901 ± 11.6 a	A 0.532 ± 9.27 b	A 0.388 ± 7.68 b	0.0016
Significant Level	N.S	N.S	N.S	

* Values=Means ± SE
** N.S = Mean No significant difference (P≤0.05).
a. b. c. small letters within a row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

In terms of the cells of in numbers of MID, according the results of table 3, there was no significant difference in numbers of MID between T1, T2 groups compared to control group in all periods except the periods of 3, 9. The T1 group was superior compared to control treatment. The results of the study are in agreement with results of (21) who noted that the treatment with arginine resulted in a decrease of the mononuclear cells proportion after two hours of treatment and then after 12 hours of treatment, the group of intravenous arginine injection and oral arginine group were significantly increased compared to control group in the blood of the awassi ewes.

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Table – 3: Effect of arginine on MID in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *0.327 ± 1.64 a	BC 0.108 ± 1.04 a	A 0.173 ± 1.14 a	N.S**
2	A 0.272 ± 1.72 a	A 0.231 ± 1.88 a	A 0.117 ± 1.34 a	N.S
3	A 0.176 ± 1.77 a	AB 0.243 ± 1.60 ab	A 0.117 ± 1.10 b	0.0524
4	A 0.222 ± 1.80 a	ABC 0.278 ± 1.45 a	A 0.198 ± 1.10 a	N.S
5	A 0.137 ± 1.57 a	A 0.187 ± 1.90 a	A 0.177 ± 1.45 a	N.S
6	A 0.124 ± 1.77 a	ABC 0.146 ± 1.34 a	A 0.221 ± 1.47 a	N.S
7	A 0.218 ± 1.54 a	ABC 0.174 ± 1.52 a	A 0.251 ± 1.38 a	N.S
8	A 0.136 ± 1.25 a	BC 0.115 ± 1.10 a	A 0.195 ± 0.985 a	N.S
9	A 0.079 ± 1.18 a	C 0.120 ± 0.985 ab	A 0.036 ± 0.857 b	0.0452
Significant Level	N.S	0.0038	N.S	

The results of table 4 showed no significant differences in the ratio of lymphocytes among the groups of T1, T2 and control in periods 1, 2, 3, 7, 8, with significant differences in the period 4, 5, 6, 9 where the group of T1 was superior compared to the control treatment, this results are in agreement with results of (18) who noted that addition of arginine has improved the humoral and cellular immunity of the animal. This increase in the proportion of lymphocytes may be attributed to the role of arginine in the development of lymphocytes, as arginine supplements promote the immune function of the body (6).

Table – 4: Effect of arginine in (Lymphocytes) in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *1.39 ± 9.58 a	A 1.16 ± 7.92 a	AB 0.271 ± 6.57 a	N.S**
2	B 1.01 ± 5.78 a	A 1.06 ± 6.70 a	C 0.516 ± 4.11 a	N.S
3	AB 0.956 ± 8.17 a	A 0.912 ± 6.50 a	BC 0.409 ± 5.44 a	N.S
4	A 1.21 ± 10.6 a	A 0.830 ± 8.41 ab	AB 0.732 ± 6.75 b	0.0306
5	A 0.676 ± 10.7 a	A 0.883 ± 7.72 b	A 0.570 ± 7.25 b	0.0061
6	AB 0.673 ± 8.65 a	A 0.511 ± 7.31 a	ABC 0.417 ± 5.62 b	0.0037
7	AB 0.944 ± 7.98 a	A 0.901 ± 7.94 a	AB 0.554 ± 6.88 a	N.S
8	AB 0.778 ± 8.80 a	A 0.670 ± 7.70 a	A 0.648 ± 7.11 a	N.S
9	A 0.744 ± 9.12 a	A 0.279 ± 6.94 b	AB 0.294 ± 5.94 b	0.0007
Significant Level	0.0245	N.S	0.0011	

Also the results of table 5 showed no significant differences in the ratio of Neutrophil among the groups of T1, T2 and control in periods 1, 2, 4, 5, 6, 7, 8, 9 except the period 3 where the group of T1 was superior compared to control treatment, the study results were in agreement with results of (22). The groups of treatment of arginine (Intravenous and muscular) showed significant superiority compared to control treatment in the number of neutrophil in the blood of awassi ewes. The increase in due may be to the effect of injection of Arginine, which has led to an increase in the growth and development of ovarian follicles, thus increasing the level of estrogen which is reflected in the proportion of white blood cells (neutrophils), This is also indicated by (23) and may also be due to higher temperatures resulting in thermal stress which leads to immune stimulation in the animal's body and increases the proportion of cells with

increased stress on animals (21).

The results of Table 6 showed no significant differences in the concentration of total protein among the groups of T1, T2 and control in period 1, 2, 3, 4, 5, 7, 9, While in the period of 6 the group of T1 was superior compared to control group, and in the period 8 the T2 group resulted in decrease of total protein concentration, this due may be to the effect of arginine because of multiple roles in the process of animal metabolism and is the basis for protein creation (24), also (25) mentioned that the arginine regulates a mechanical target for the signaling pathway within the cell that has important roles in the synthesis of proteins, cell proliferation, and cytoskeletal

Table – 5: Effect of arginine in (Neutrophil) in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	BC *0.556 ± 2.70 a	C 0.400 ± 1.48 a	B 0.375 ± 2.24 a	N.S**
2	A 1.21 ± 6.21 a	A 0.614 ± 4.20 a	A 0.601 ± 3.25 a	N.S
3	BC 0.413 ± 2.98 a	BC 0.286 ± 2.20 ab	BCD 0.246 ± 1.42 b	0.0114
4	BC 0.291 ± 1.98 a	BC 0.291 ± 1.64 a	CD 0.198 ± 1.04 a	N.S
5	BC 0.219 ± 2.08 a	BC 0.281 ± 2.37 a	BCD 0.226 ± 1.61 a	N.S
6	BC 0.408 ± 2.50 a	BC 0.221 ± 2.04 a	BCD 0.260 ± 1.87 a	N.S
7	B 0.500 ± 3.24 a	B 0.389 ± 2.65 a	BC 0.335 ± 1.94 a	N.S
8	C 0.226 ± 1.45 a	C 0.238 ± 1.28 a	CD 0.247 ± 1.02 a	N.S
9	C 0.134 ± 1.30 a	C 0.260 ± 1.34 a	D 0.085 ± 0.885 a	N.S
Significant Level	0.0001	0.0001	0.0001	

modification. The results are disgree with results of (26) in his study on pigs, which showed that no significant differences in total protein concentration under influence of arginine treatments. The results of Table 7 showed no significant differences in albumin concentration among the groups of T1 and T2 and control in all periods except for period of 8 where albumin concentration decreased in group T2. (27) showed that the albumin value was significantly increased when vaginal sponges were putted in sheep and goats. The albumin value was 2.99 gm.dl before treatment while become 3.79 gm.dl after treatment.

Table – 6: Effect of arginine in total protein in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *0.680 ± 6.71 a	BCD 0.617 ± 7.00 a	A 0.553 ± 6.85 a	N.S**
2	A 0.202 ± 5.42 a	BCD 0.436 ± 7.00 a	A 0.737 ± 7.85 a	N.S
3	A 1.05 ± 7.14 a	AB 0.642 ± 8.35 a	A 1.04 ± 7.57 a	N.S
4	A 0.521 ± 7.71 a	ABC 0.532 ± 7.71 a	A 0.571 ± 7.42 a	N.S
5	A 0.420 ± 7.28 a	ABC 0.480 ± 7.57 a	A 0.723 ± 9.00 a	N.S
6	A 0.808 ± 8.28 a	BCD 0.528 ± 6.57 ab	A 0.508 ± 5.85 b	0.0385
7	A 0.554 ± 7.21 a	CD 0.653 ± 6.21 a	A 0.368 ± 7.57 a	N.S
8	A 0.609 ± 6.98 a	D 0.285 ± 5.28 b	A 0.577 ± 8.00 a	0.0051
9	A 0.769 ± 7.85 a	A 0.865 ± 9.28 a	A 0.848 ± 8.07 a	N.S
Significant Level	N.S	0.0006	N.S	

* Values=Means ± SE
** N.S = Mean No significant difference (P≤0.05).
a. b. c. small letters within a row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

There were no significant differences from Table 8 among the groups T1, T2 and control in the concentration of globulin in all periods except period 6, where observed the superiority of group T1 was observed compared to control treatment. The superiority of T1 may be attributed to the role of amino acid arginine with increased vitality and activity of goats treated with Arginine, which leads to an increase in the immune status of the body and resistance against diseases, where (28) noted that there isan action mechanism of arginine with immunity, where receptors of arginine are present on the surfaces of epithelial cells of thymus glands. Also many studies which conducted on mammals suggest that arginine supplementation increases the reproduction and functional interactions of lymphocytes, thymus glands and spleen (29). These results were in agreement with results of (30) who used intravenous injection of the arginine, which led to a significant increase in the concentration of globulin in the animal's body.

Table – 7: Effect of arginine in albumin in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *0.229 ± 3.071 a	A 0.308 ± 3.000 a	A 0.177 ± 3.385 a	N.S.**
2	A 0.193 ± 3.342 a	A 0.171 ± 3.342 a	A 0.202 ± 3.428 a	N.S
3	A 0.218 ± 3.385 a	A 0.260 ± 3.357 a	A 0.137 ± 3.428 a	N.S
4	A 0.231 ± 3.857 a	A 0.278 ± 3.685 a	A 0.220 ± 3.071 a	N.S
5	A 0.177 ± 3.542 a	A 0.142 ± 3.428 a	A 0.232 ± 3.271 a	N.S
6	A 0.245 ± 3.628 a	A 0.182 ± 2.942 a	A 0.098 ± 3.585 a	N.S
7	A 0.268 ± 3.142 a	A 0.232 ± 3.100 a	A 0.329 ± 3.814 a	N.S
8	A 0.150 ± 3.542 a	A 0.089 ± 2.842 b	A 0.144 ± 3.742 a	0.0003
9	A 0.443 ± 3.871 a	A 0.316 ± 3.571 a	A 0.397 ± 4.000 a	N.S
Significant Level	N.S	N.S	N.S	

* Values=Means ± SE
** N.S = Mean No significant difference (P≤0.05).
a. b. c. small letters within a row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

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Table – 8: Effect of arginine in globulin in local goats

Days	Treatments			Significant Level
	T1	T2	T3	
1	A *0.943 ± 4.643 a	ABCD 0.617 ± 4.000 a	A 0.597 ± 3.471 a	N.S.**
2	A 0.298 ± 2.085 a	BCD 0.359 ± 3.657 a	A 0.751 ± 4.428 a	N.S
3	A 1.006 ± 3.900 a	AB 0.715 ± 5.000 a	A 1.081 ± 4.143 a	N.S
4	A 0.618 ± 3.857 a	ABCD 0.533 ± 4.028 a	A 0.548 ± 4.928 a	N.S
5	A 0.355 ± 3.742 a	ABC 0.572 ± 4.285 a	A 0.769 ± 5.728 a	N.S
6	A 0.790 ± 4.657 a	BCD 0.469 ± 3.628 ab	A 0.449 ± 2.271 b	0.0341
7	A 0.620 ± 4.071 a	CD 0.601 ± 3.114 a	A 0.478 ± 3.757 a	N.S
8	A 0.605 ± 3.442 a	D 0.225 ± 2.442 a	A 0.536 ± 4.257 a	N.S
9	A 0.930 ± 3.986 a	A 0.742 ± 5.714 a	A 1.034 ± 4.071 a	N.S
Significant Level	N.S	N.S	N.S	

* Values=Means ± SE

** N.S = Mean No significant difference (P≤0.05).

a. b. c. small letters within a row indicate significant differences between the treatments. The large letters within column indicate that significant differences between the sampling days within the ones treatment at significant level (P≤0.05).

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